二代测序
Next-Generation Sequencing

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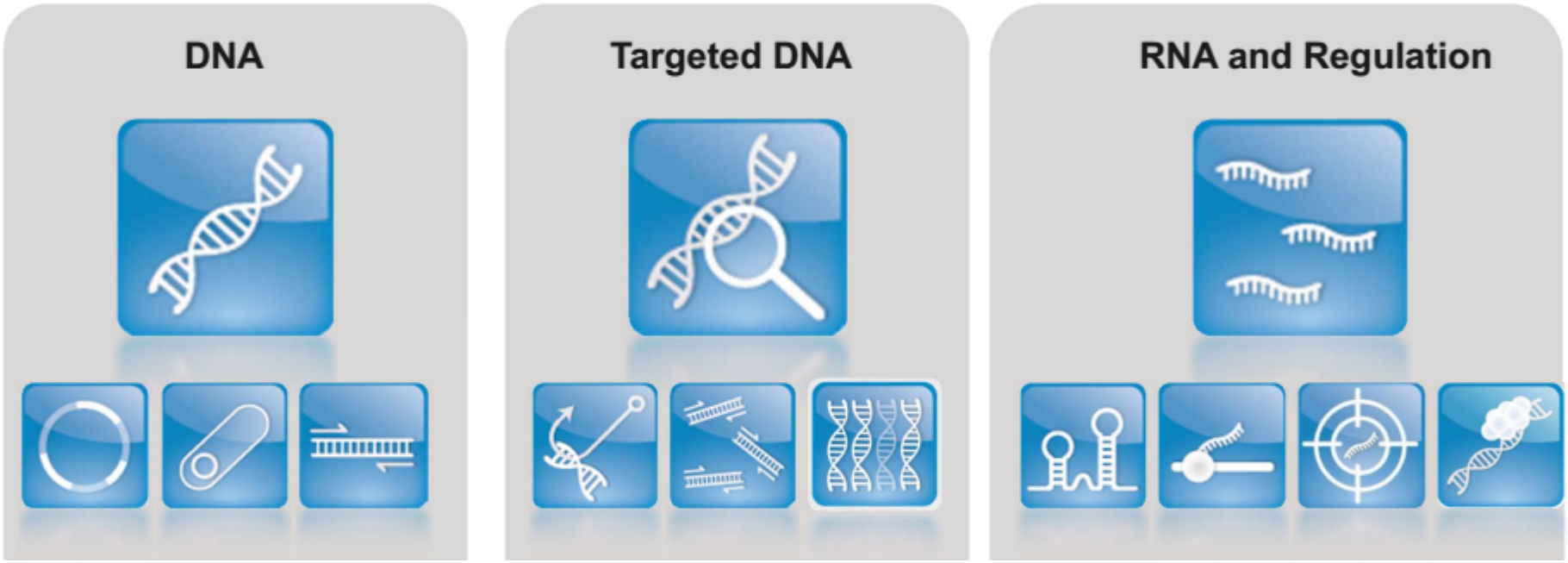
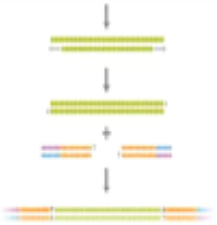
NGS应用



Illumina Sequencing Workflow
Illumina 测序流程

1

Library Preparation
文库制备



2

Cluster Generation
簇生成

cBot
MiSeq
NextSeq
HiSeq 2500-Rapid

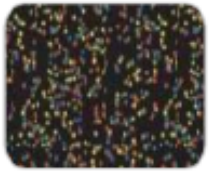
3

Sequencing
测序

HiSeq
HiScan SQ
GA IIx
MiSeq
NextSeq

4

Data Analysis
数据分析



ICS/RTA
CASAVA
MSR
BaseSpace

文库制备

TruSeq DNA PCR Free文库制备实验流程

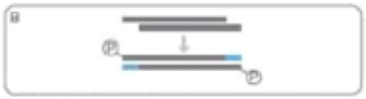
起始DNA质检及定量

➡ 用荧光染料法定量DNA

DNA打断

➡ 利用Covaris仪器中的设定打断DNA至两种不同插入片段大小
- 350 bp 或 550 bp
- DNA打碎后立即进行一次纯化（磁珠法）

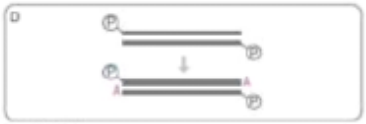
末端修复



片段筛选（磁珠法）

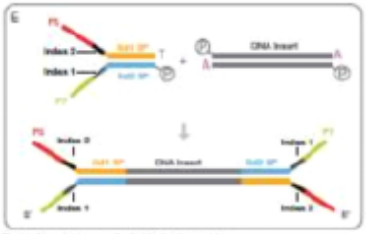
➡ 磁珠法片段筛选
- 该步骤对片段大小分布和文库产出至关重要

加A尾



➡ 加完A尾后必须进行酶的热失活
- 减少接头二聚体的形成

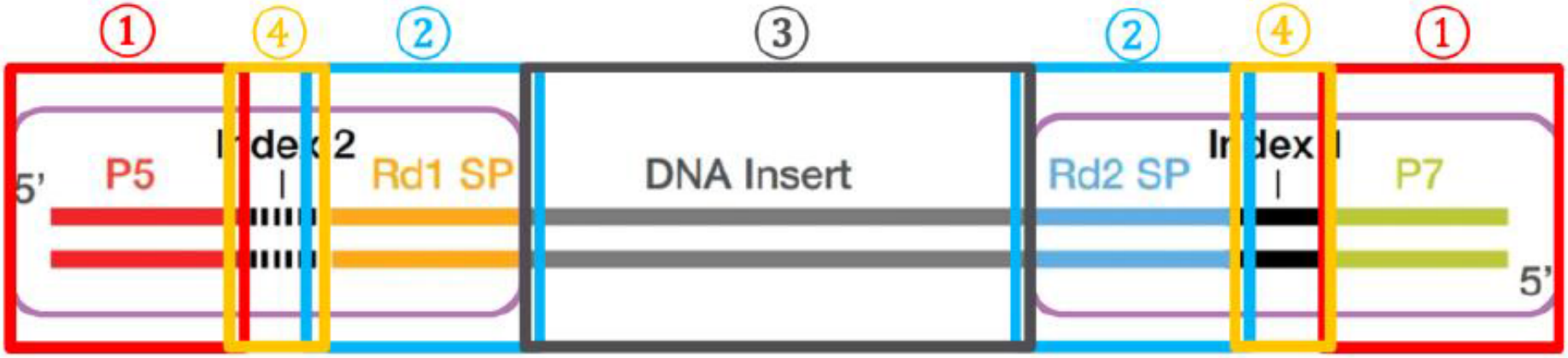
接头连接



➡ 连接反应的效率与起始输入DNA量是否准确密切相关

文库质检及定量

➡ 只能用qPCR法进行定量



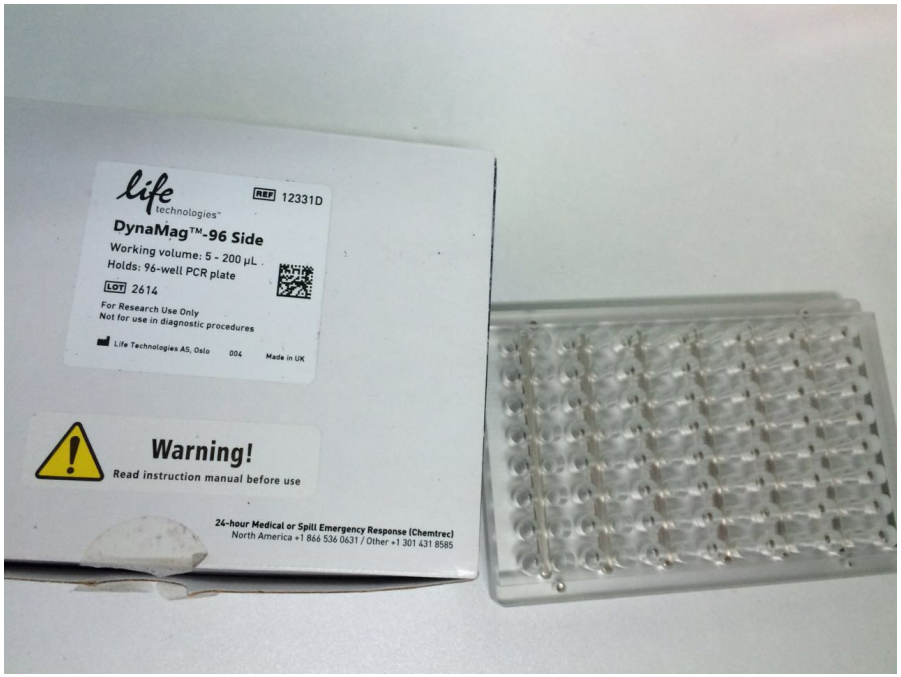
双端标签文库

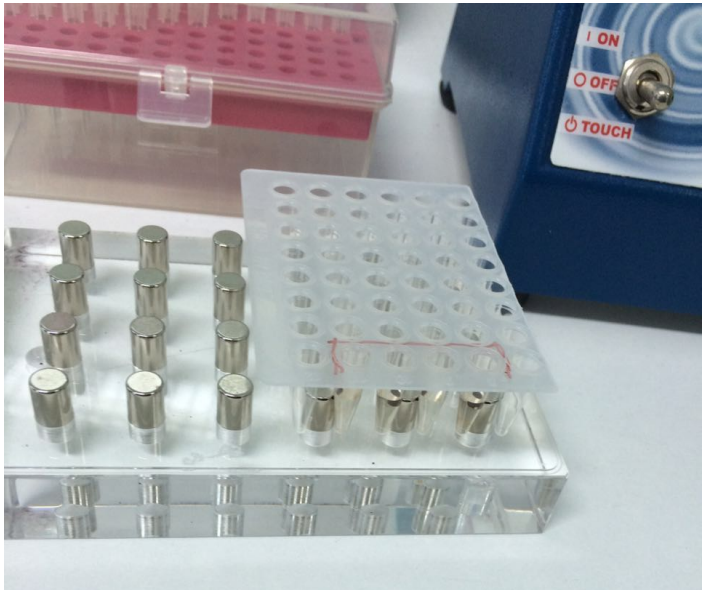
① 与流动槽（Flow Cell）结合的区域
② Read 1和Read2测序引物结合的区域
③ 插入片段
④ 标签序列区域（Index）

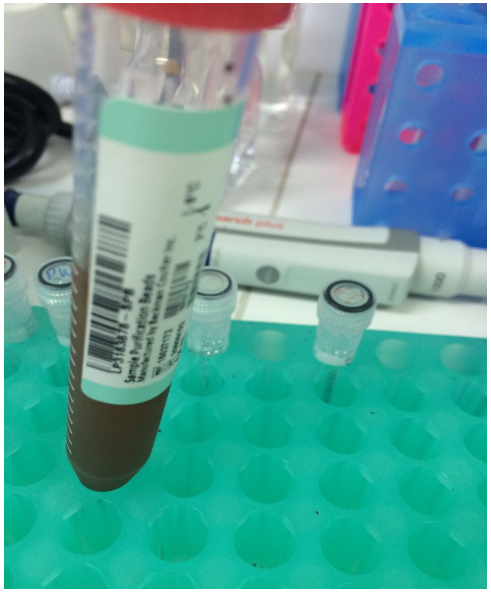
单边index 6个碱基；双边index 8个碱基，最多可以做384个samples

文库制备的目的是在需要测序的DNA片段两端加上能够与测序仪配合的接头序列（Multiplexed, SR, PE）


Qubit® 2.0
Fluorometer
Choose Your Assay:
dsDNA
High Sensitivity
dsDNA
Broad Range
ssDNA
RNA
Protein
Home
Standards
Sample
Data
invitrogen


life technologies
REF 12331D
DynaMag™-96 Side
Working volume: 5 - 200 µL
Holds: 96-well PCR plate
LOT 2614
For Research Use Only
Not for use in diagnostic procedures
Life Technologies AS, Oslo
004
Made in UK
Warning!
Read instruction manual before use
24-hour Medical or Spill Emergency Response (Chemtrec)
North America +1 866 536 0631 / Other +1 301 431 8585


I ON
O OFF
TOUCH




Covaris
Focused-ultrasonicator
M220

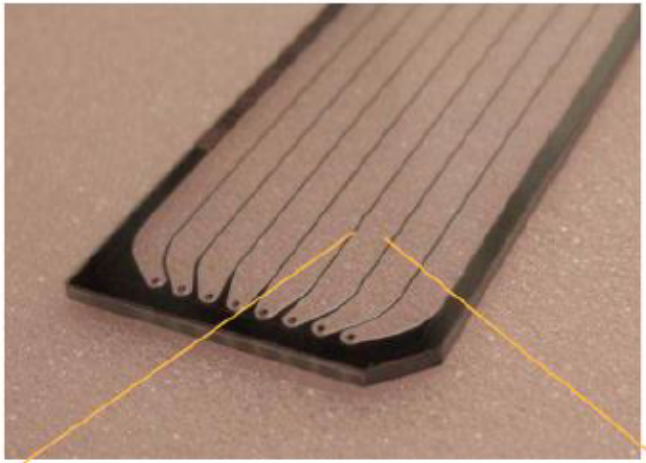
簇生成、测序

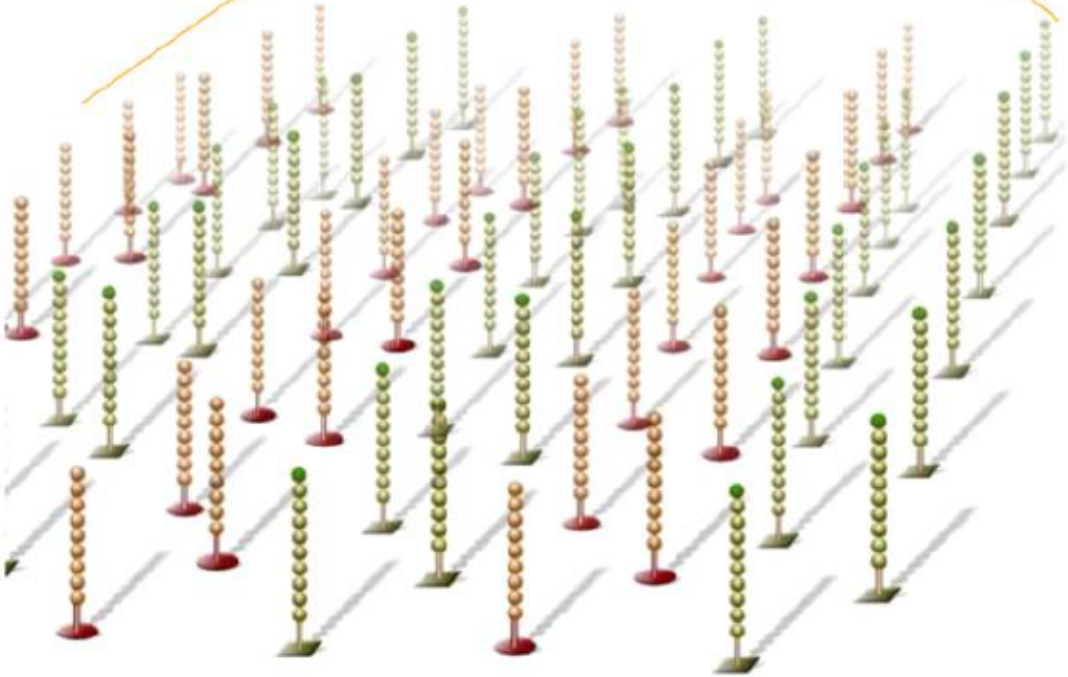
What is a Flow Cell?
流动槽(Flow Cell) 是什么?

A flow cell is a thick glass slide with channels or lanes
一种含有通道的厚玻璃片

Cluster generation occurs on a flow cell
簇生成在流动槽（flow cell）上完成

Each lane is randomly coated with a lawn of oligos that are complementary to library adapters
每条通道中都随机植入了能与文库接头互补结合的大量短DNA片段

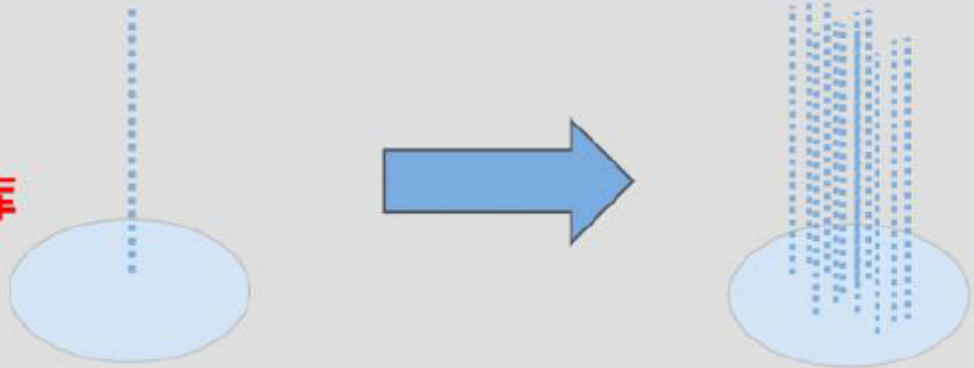




簇生成

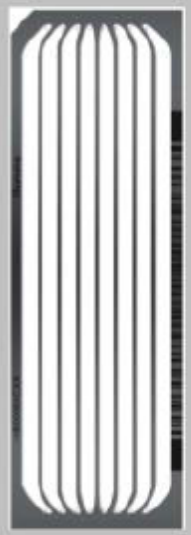
(单个荧光太弱无法捕捉，故需要簇生成增强荧光)

Single DNA Library
单个DNA文库分子



Amplified Clonal Cluster
扩增后的DNA克隆簇

cBot

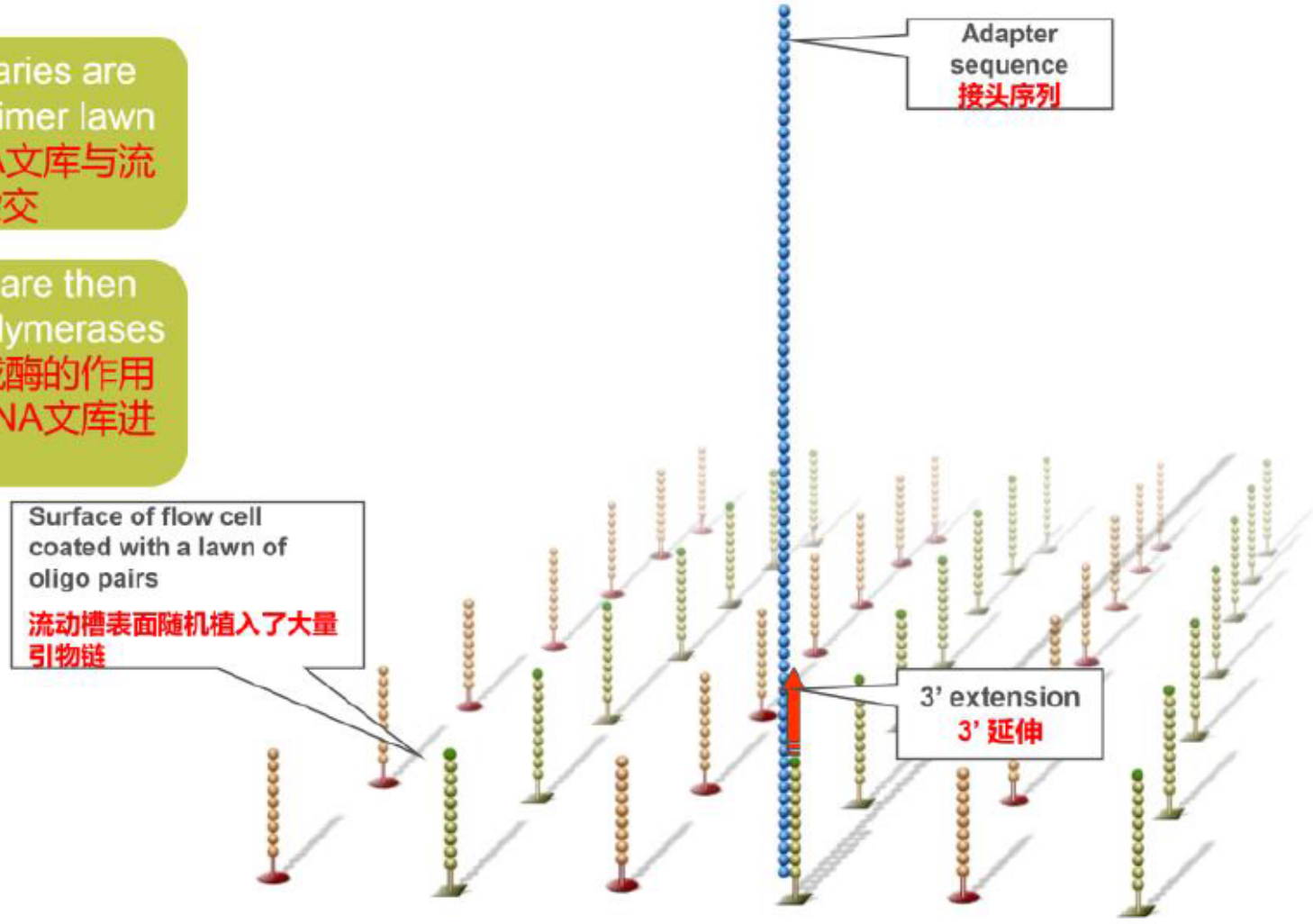


Sequencer

Hybridize Fragment & Extend
片段杂交与延伸

Single DNA libraries are hybridized to primer lawn
变性后单链DNA文库与流动槽上的引物杂交

Bound libraries are then extended by polymerases
随后在DNA合成酶的作用下，结合上的DNA文库进行延伸

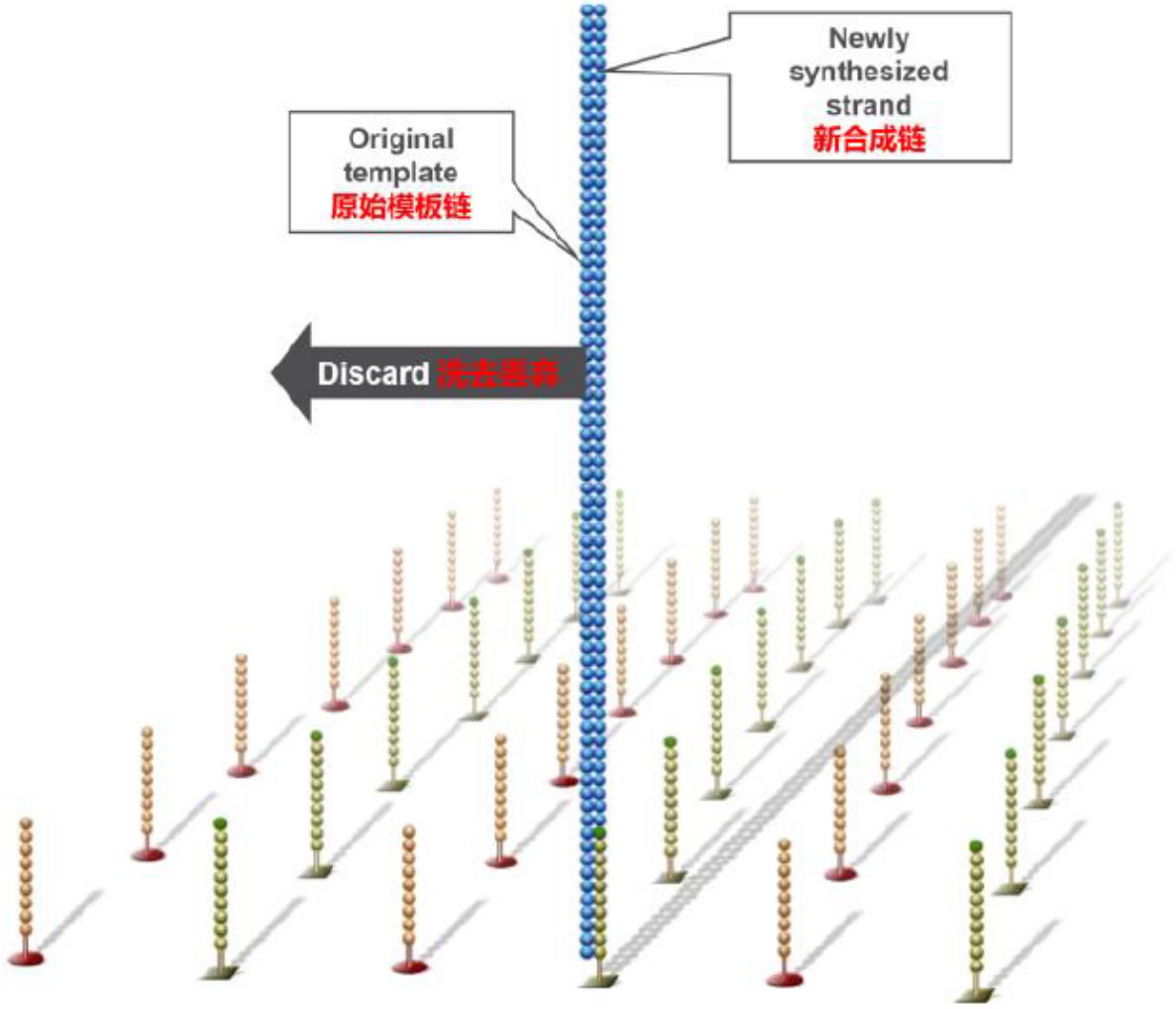


Denature Double-Stranded DNA
双链DNA变性

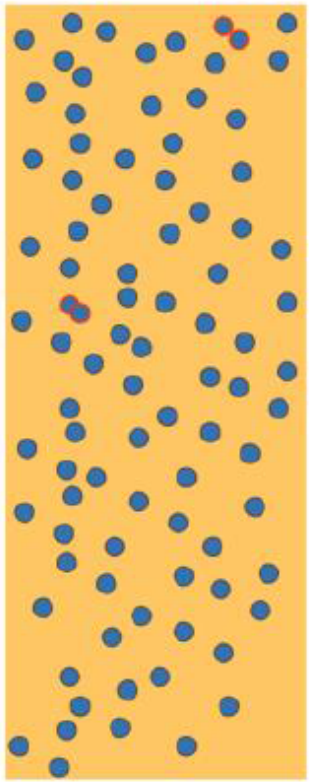
Double-stranded molecule is denatured
双链DNA分子被变性

Original template washed away
原始的模板链被洗去

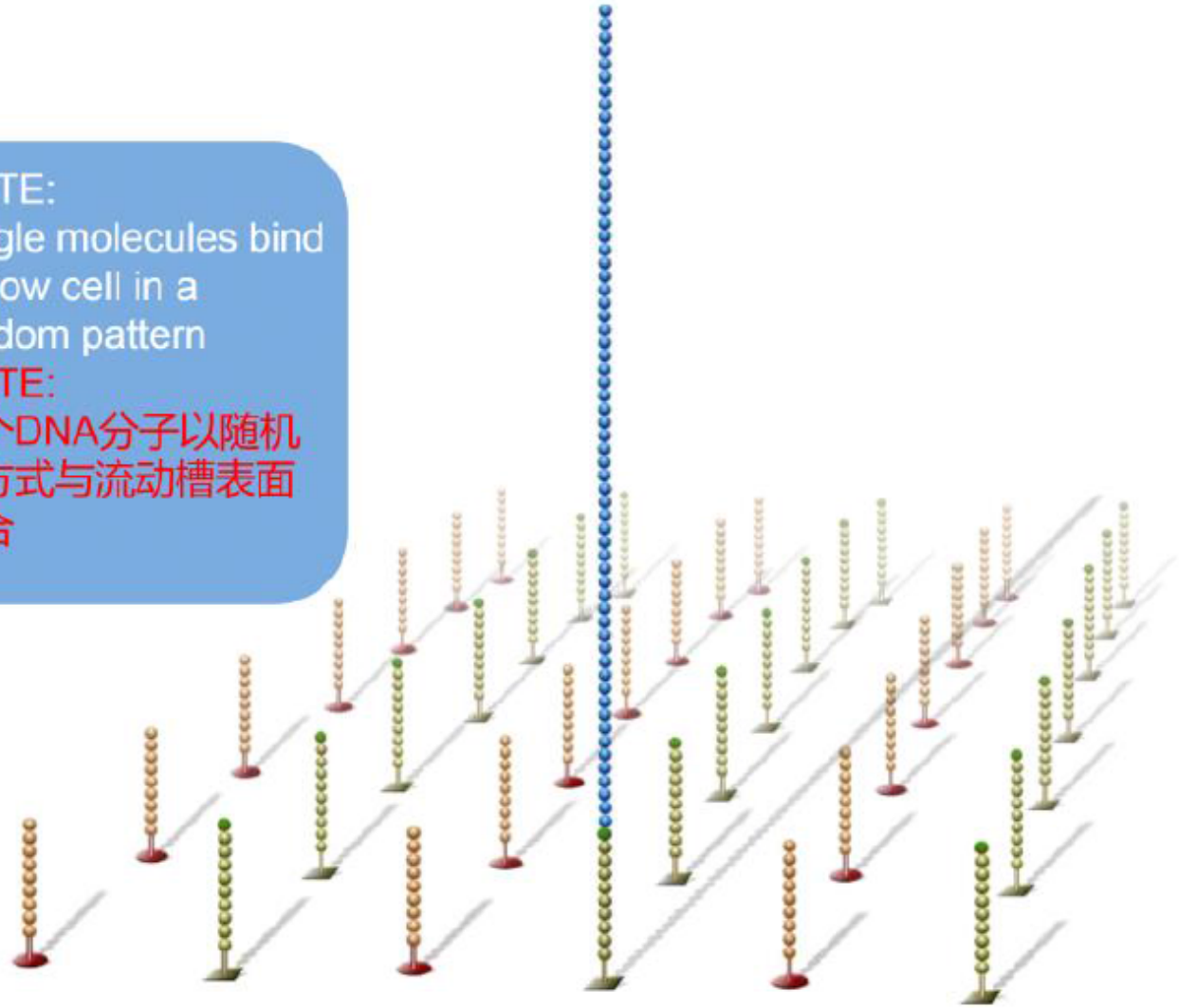
Newly synthesized strand is covalently attached to flow cell surface
新合成的DNA链以共价键连接的方式结合在流动槽表面



Single-Stranded DNA
单链DNA



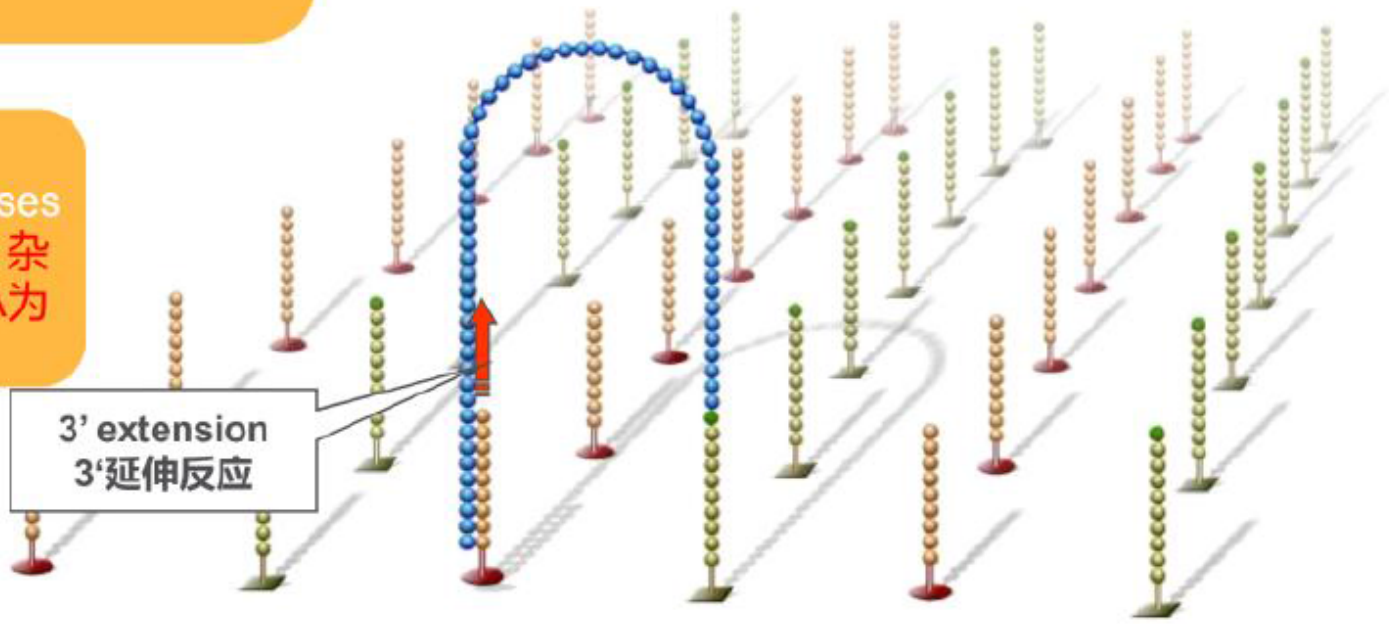
NOTE:
Single molecules bind to flow cell in a random pattern
NOTE:
单个DNA分子以随机的方式与流动槽表面结合



Bridge Amplification
桥式PCR扩增

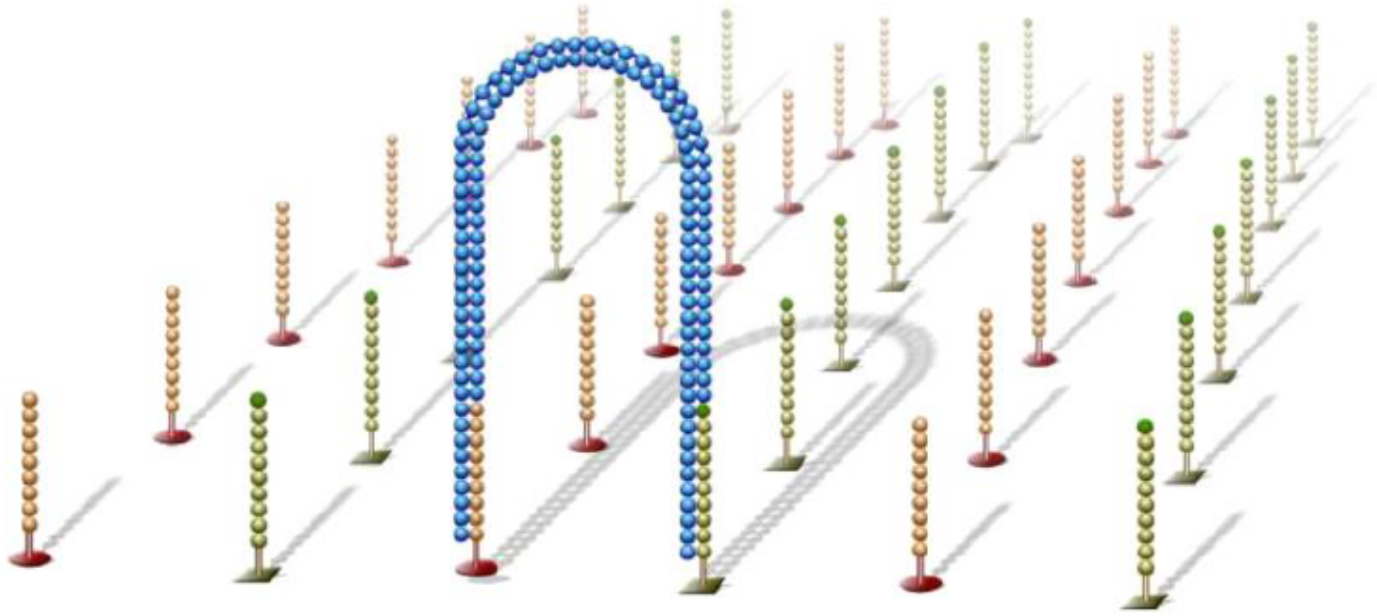
Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent, complementary primer
共价键结合在流动槽表面的单链DNA分子与其附近的互补引物杂交，整条DNA分子折叠后形成一种类似于桥的结构。

Hybridized primer is extended by polymerases
在DNA合成酶作用下，杂交后的引物以单链DNA为模板进行延伸



Bridge Amplification
桥式PCR扩增

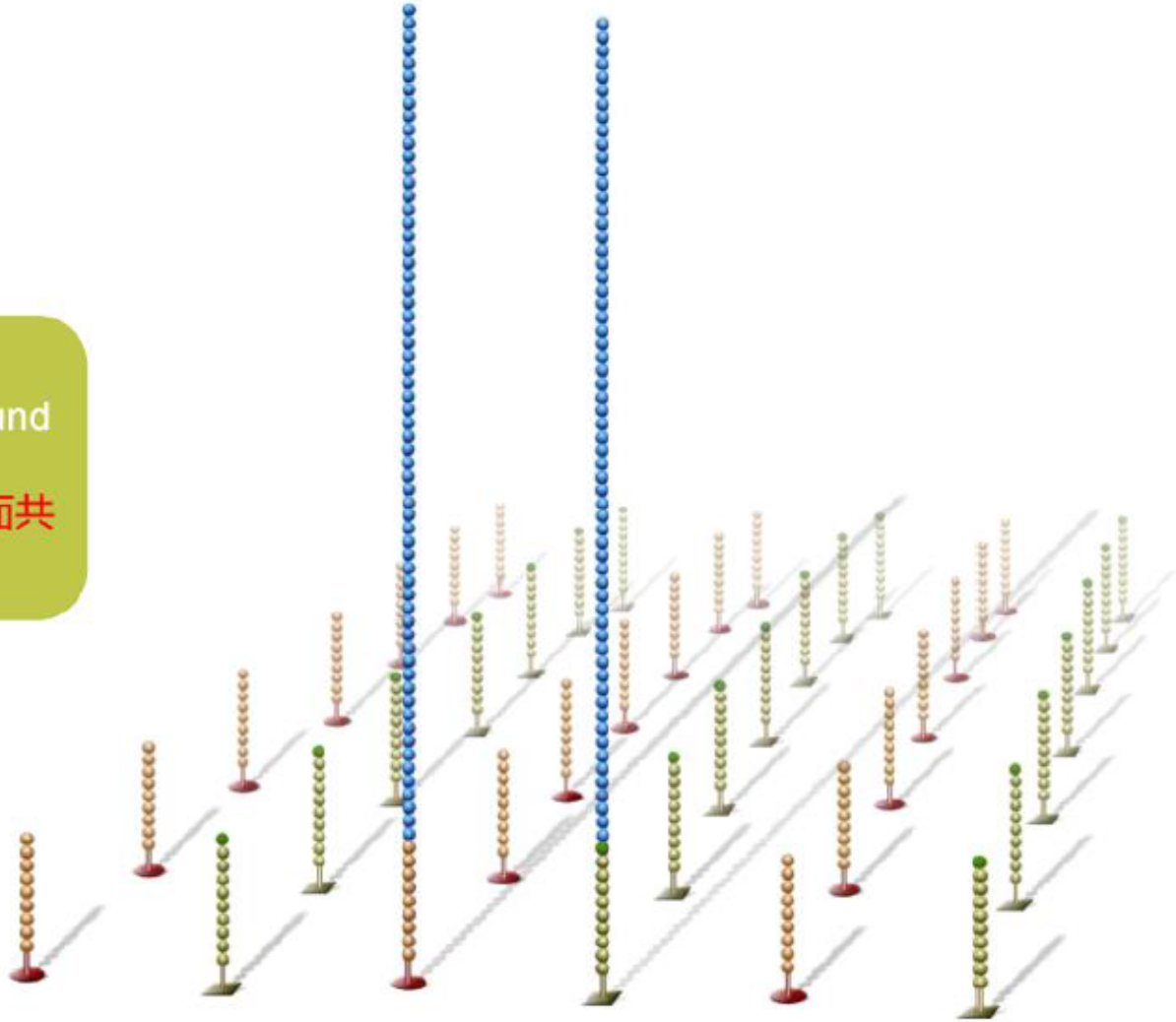
Double-stranded bridge is formed
延伸完成后形成双链DNA桥式结构



Denature Double-Stranded Bridge
双链DNA桥式结构变性

Double-stranded bridge is denatured
桥式结构的双链DNA被变性

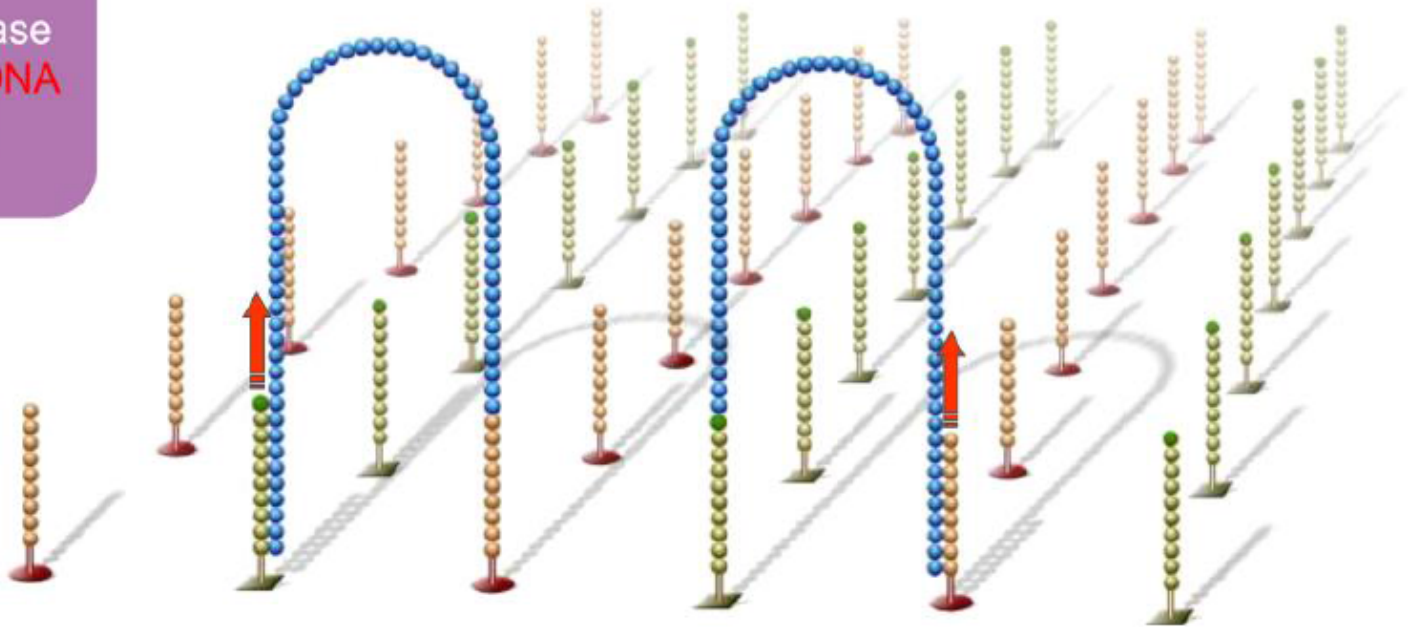
Result:
Two copies of covalently bound single-stranded templates
结果：形成两条与流动槽表面共价键结合的DNA模板



Bridge Amplification
桥式PCR扩增

Single-stranded molecules flip over to hybridize to adjacent primers
单链DNA分子再一次折叠后与附近的引物杂交结合

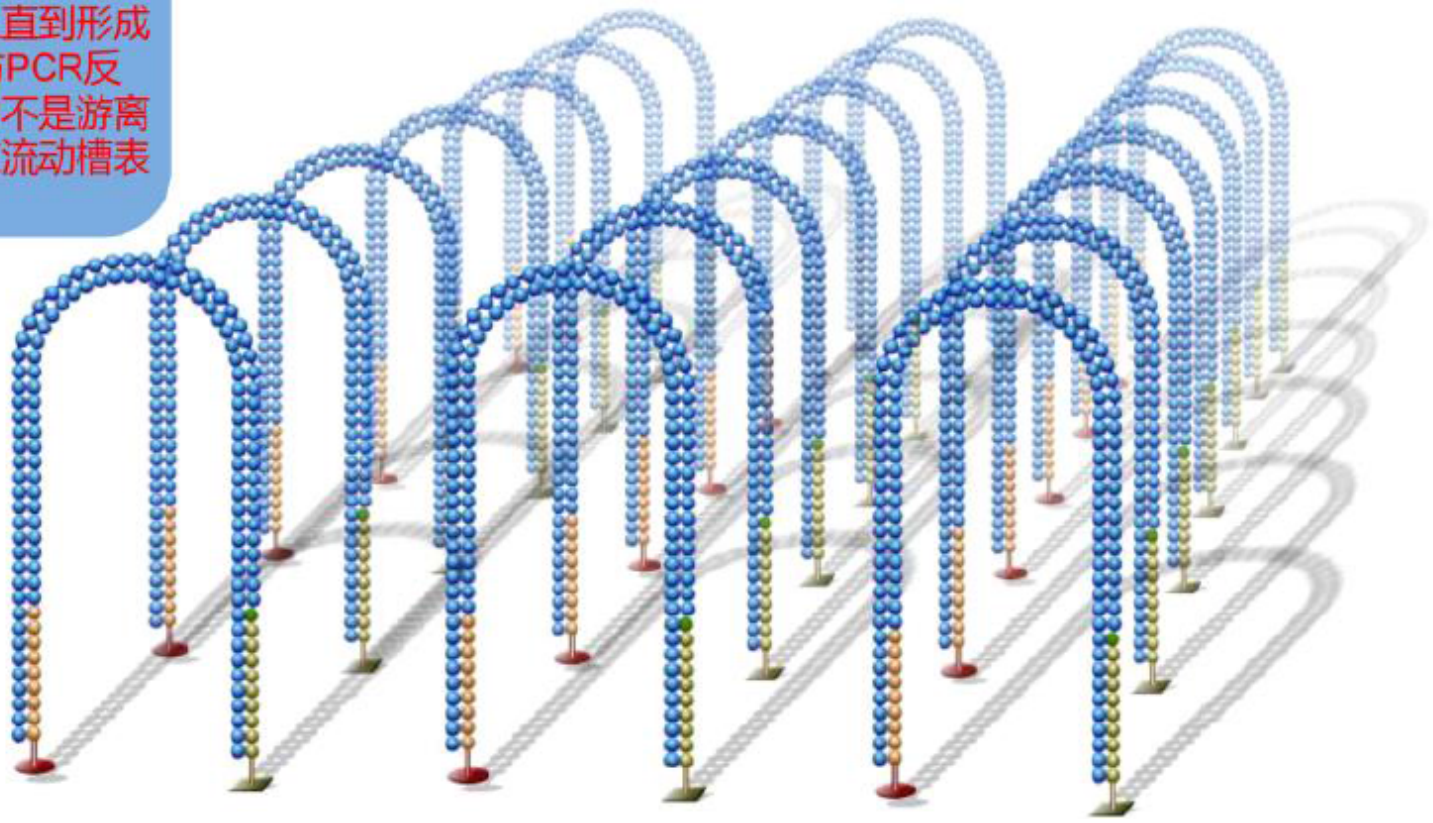
Hybridized primer is extended by polymerase
杂交后的引物再次在DNA合成酶的作用下延伸



Bridge Amplification
桥式PCR扩增

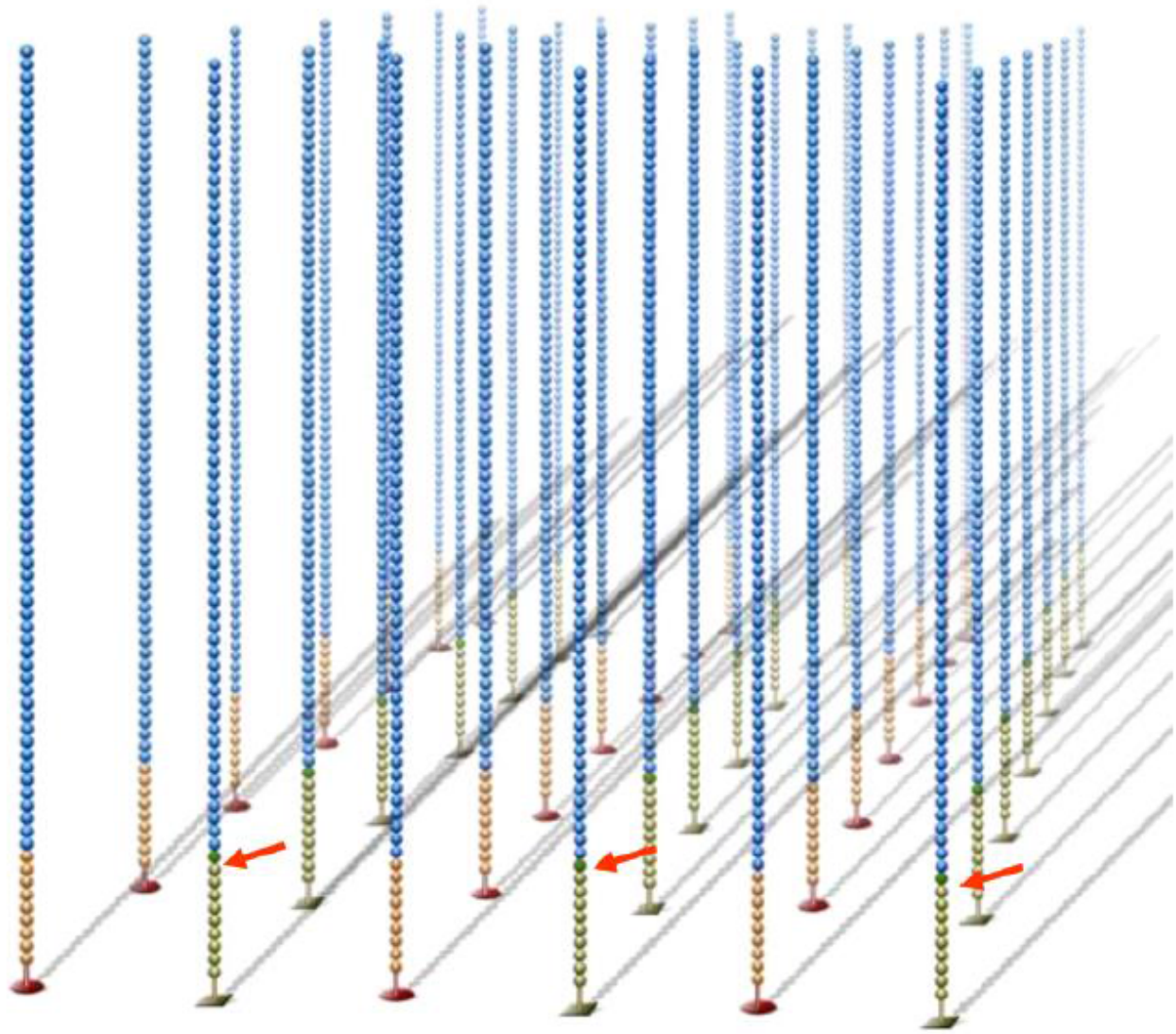
Bridge amplification cycle is repeated until multiple bridges are formed

桥式扩增不断重复发生直到形成数量足够的DNA桥（与PCR反应类似，区别在于引物不是游离在溶液中，而是固定在流动槽表面）



Linearization
线性化

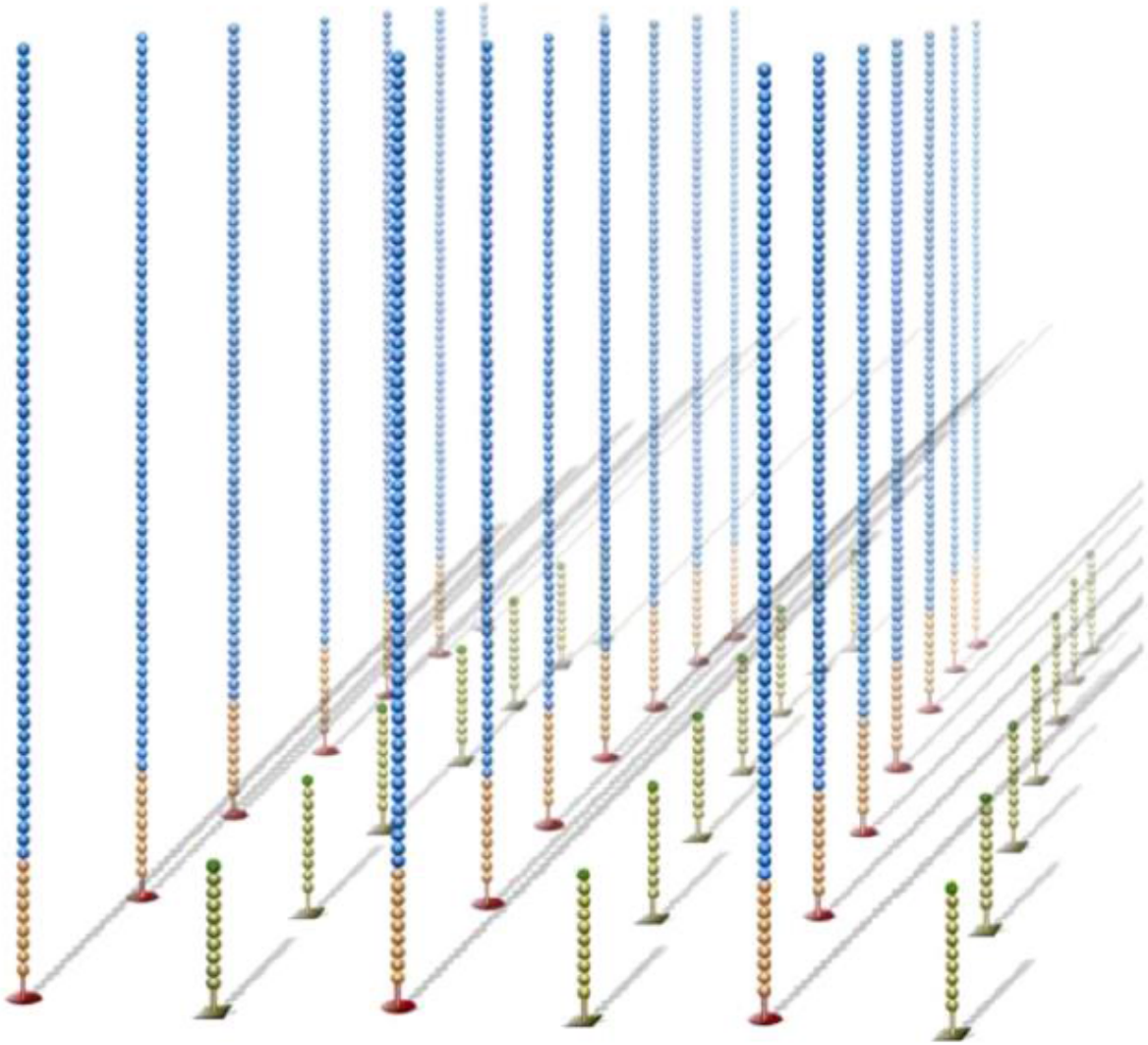
dsDNA bridges are denatured
双链DNA变性后，解开桥式结构，变成线性化的单链DNA



Reverse Strand Cleavage
反链切除

Reverse strands are cleaved and washed away, leaving a cluster with forward strands only

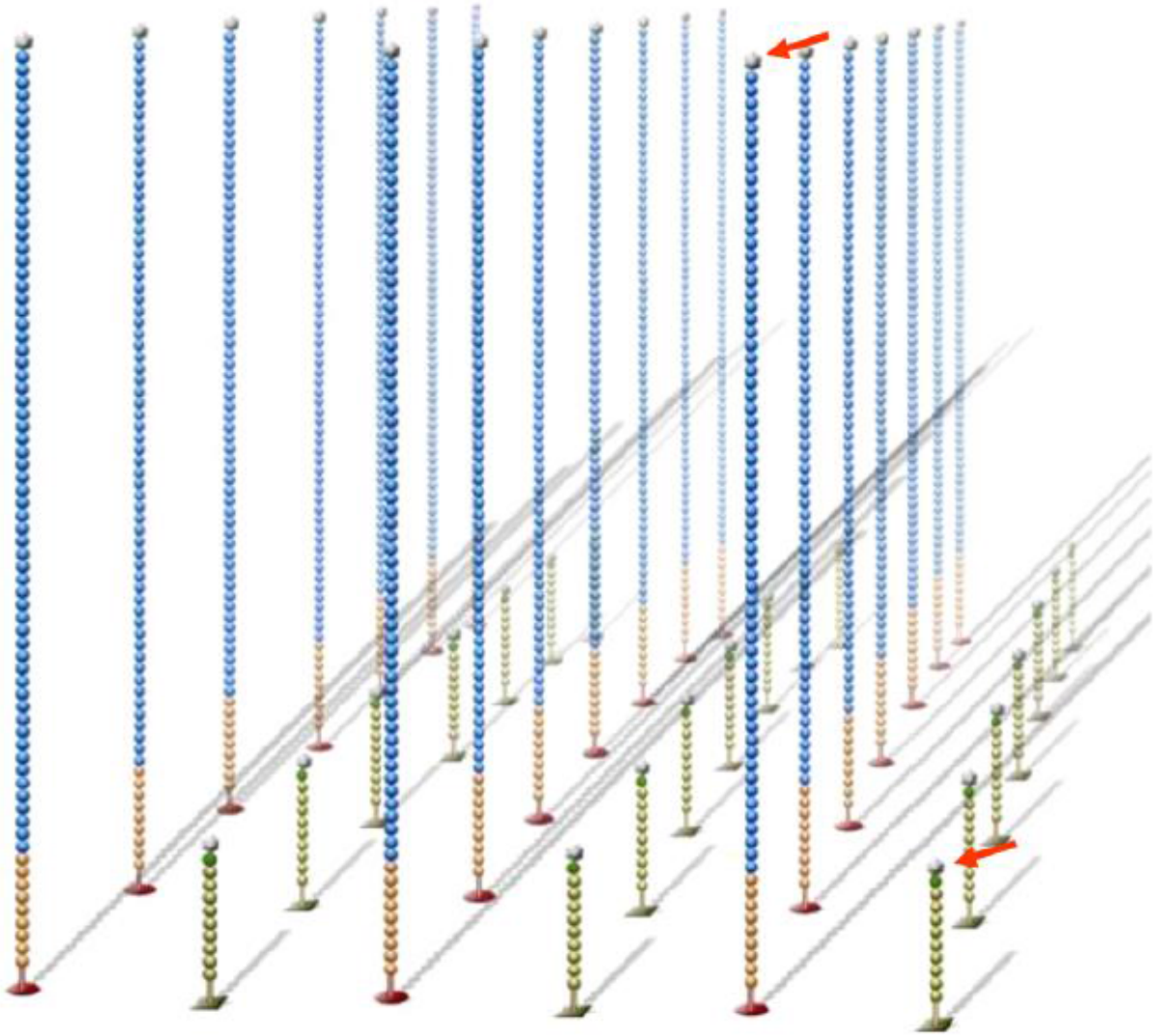
与流动槽表面结合的DNA反链被切除并洗去，只留下正链，形成包含均一单链的DNA簇



Blocking
DNA链封闭

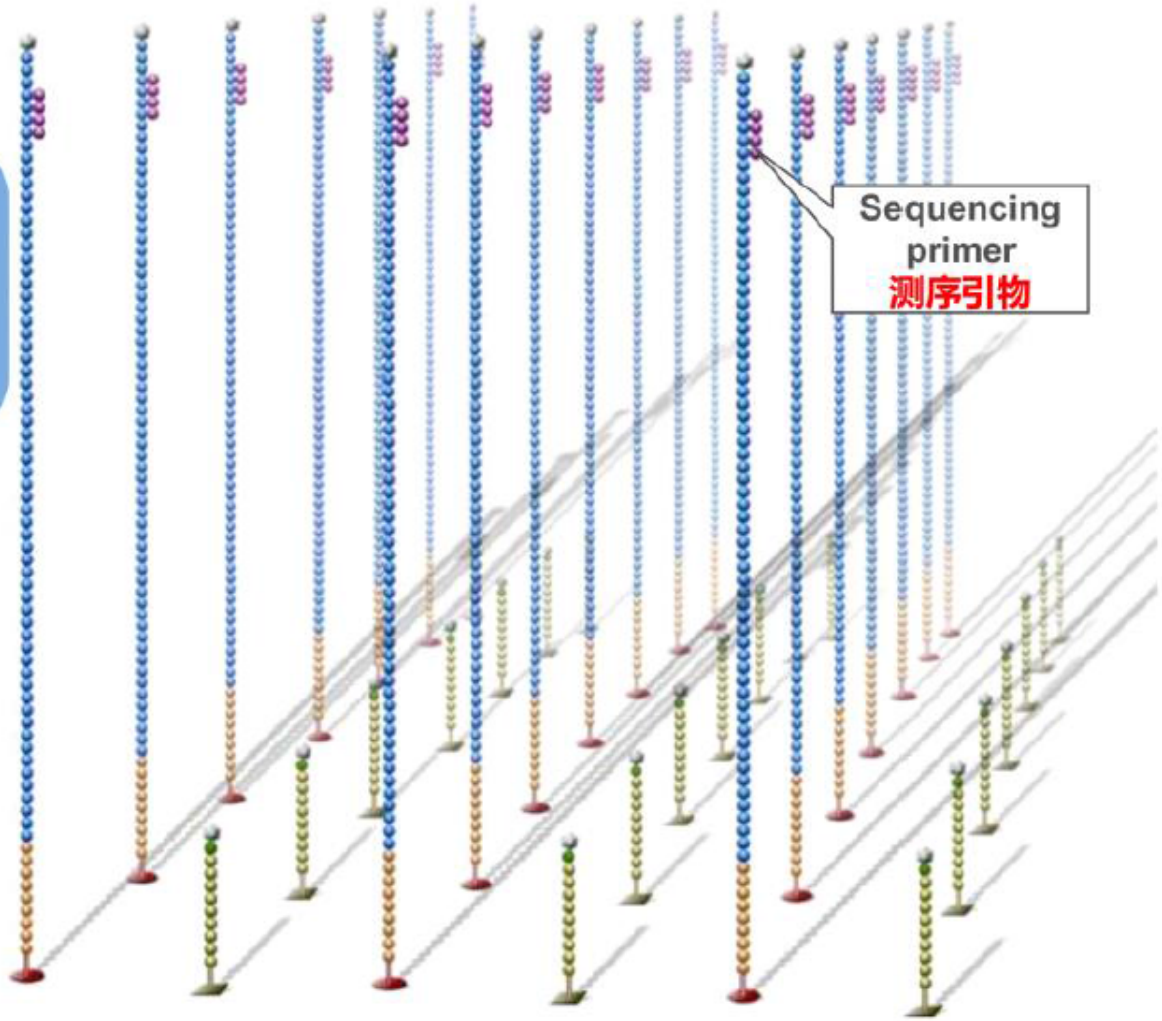
Free 3’ ends are blocked to prevent unwanted DNA priming

为了防止后续测序过程中不必要的DNA延伸，对流动槽上结合的所有DNA分子的3’端进行封闭

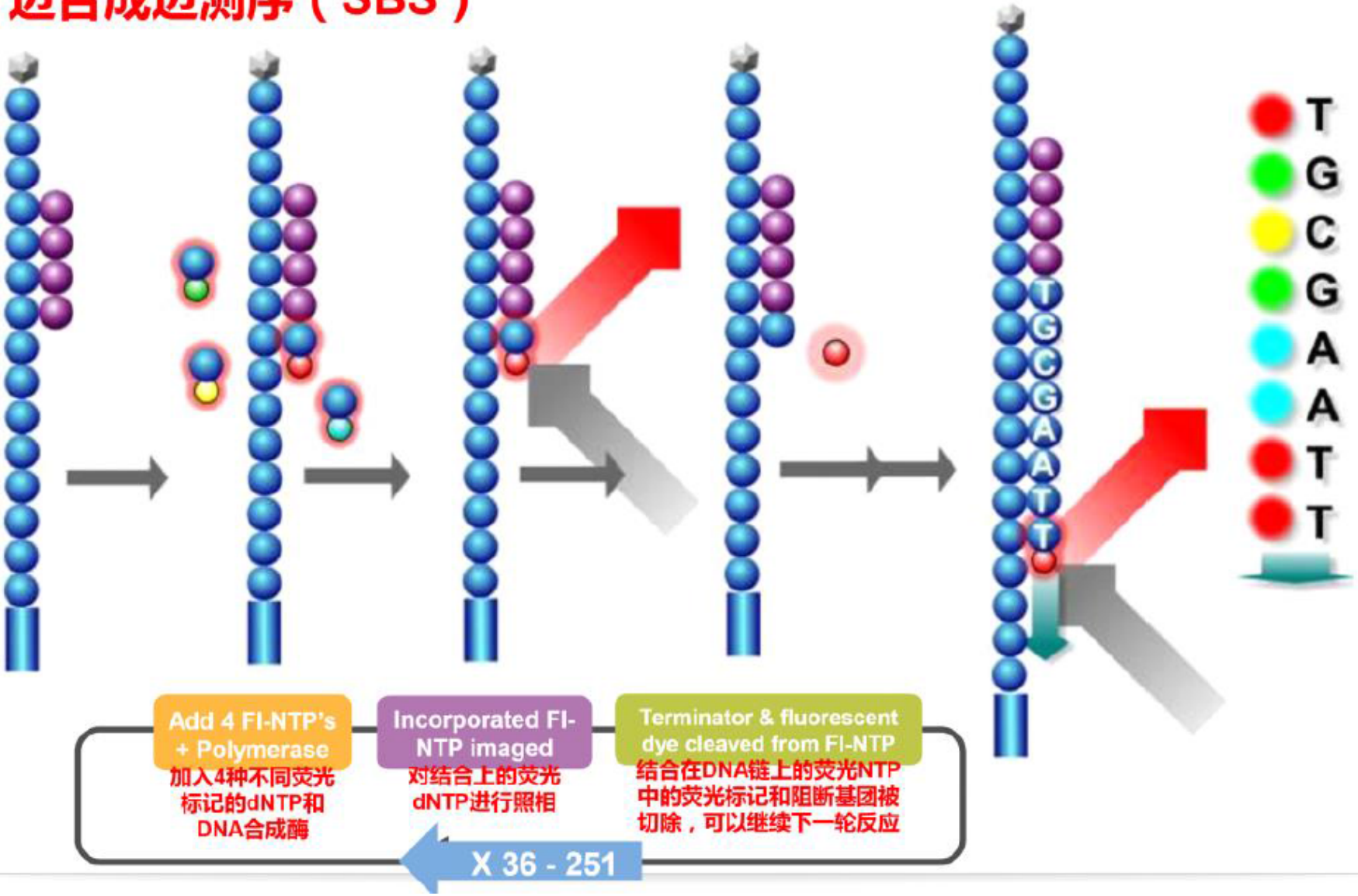


Read 1 Primer Hybridization
Read 1引物杂交

Sequencing primer is hybridized to adapter sequence
将Read 1测序引物加入流动槽，使其与待测DNA分子的接头序列结合



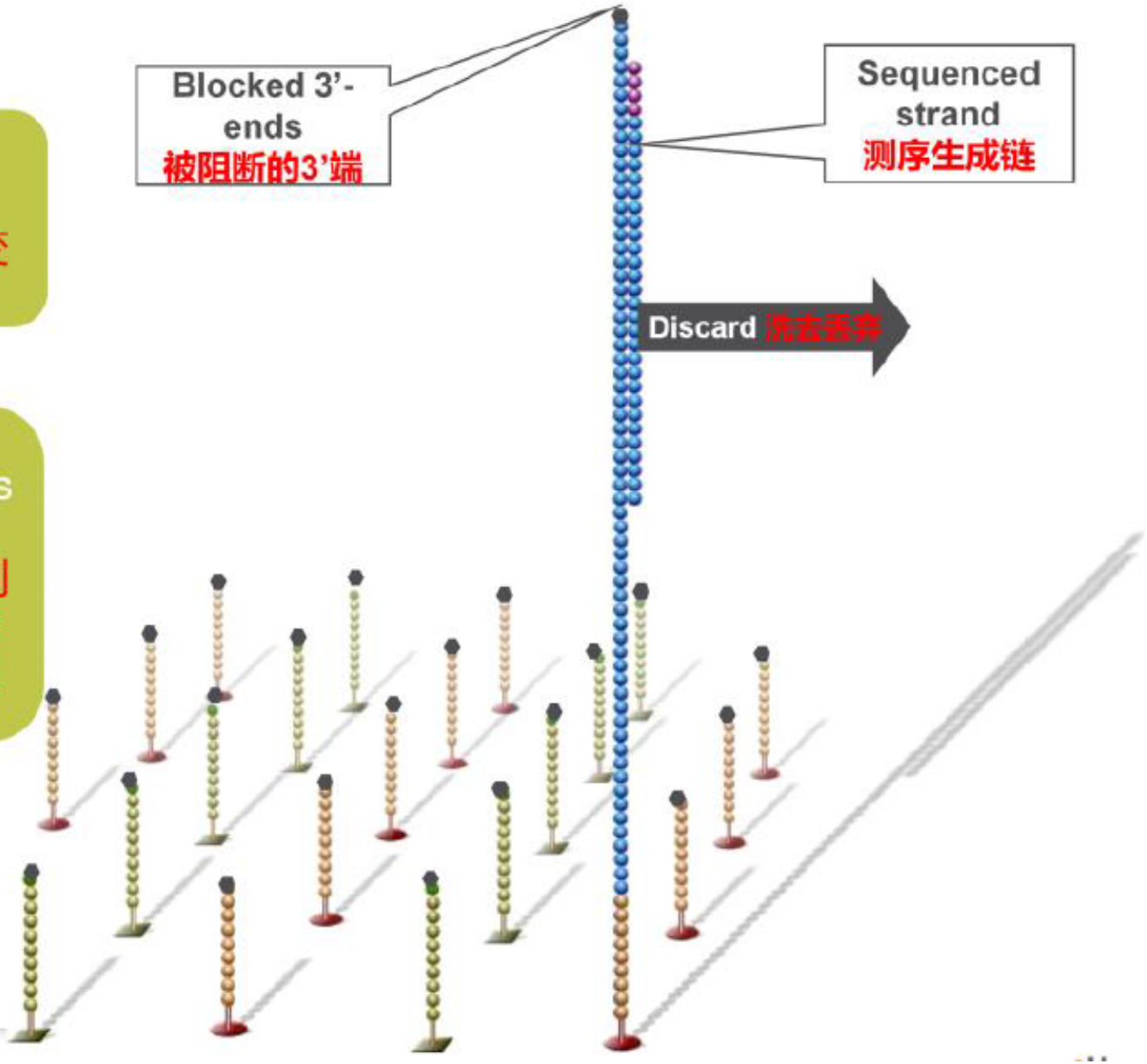
Sequencing By Synthesis
边合成边测序（SBS）



Paired End Sequencing双末端测序

Sequenced strand is stripped off
测序反应生成的片段被变性洗去

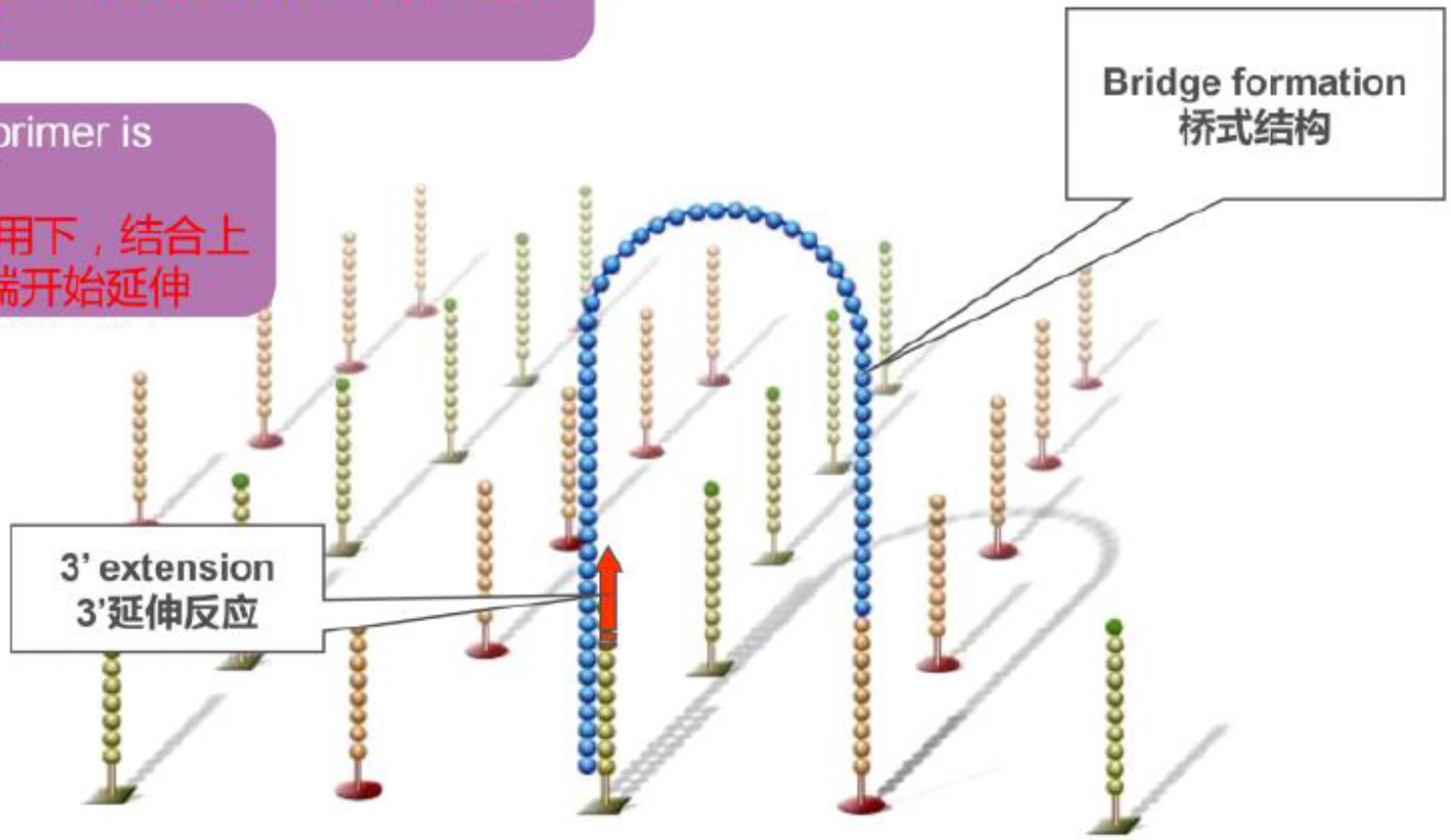
3'-ends of template strands and lawn primers are unblocked
与流动槽结合的DNA序列上3'端阻断被去除（同时也去除引物丛上的3'端阻断）



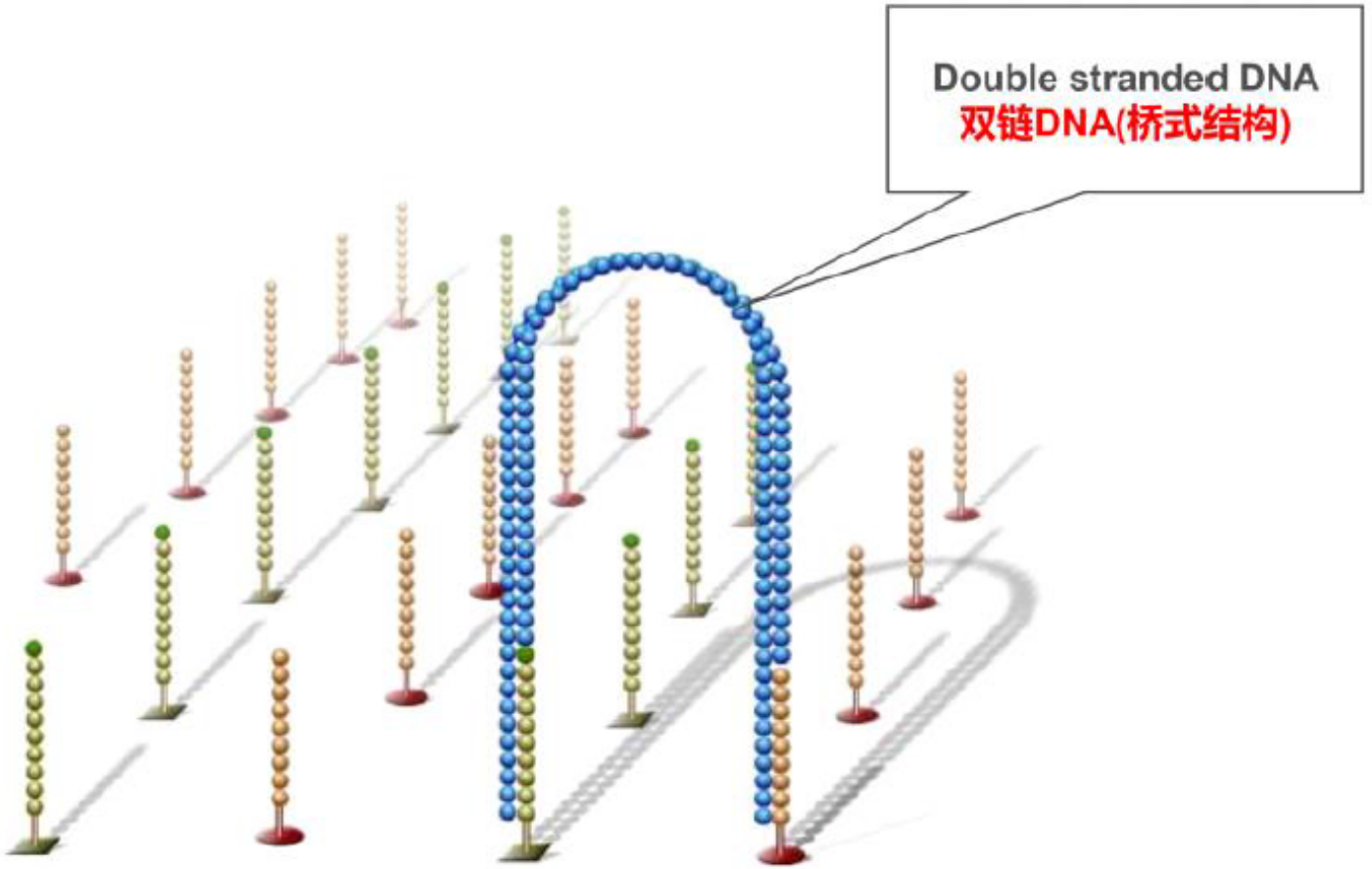
Paired End Sequencing
双末端测序

Single-stranded template loops over to form a bridge by hybridizing with a lawn primer
DNA单链折叠后与其附近的引物丛杂交结合形成新的桥式结构

3'-ends of lawn primer is extended
在DNA合成酶作用下，结合上模板的引物丛3'端开始延伸

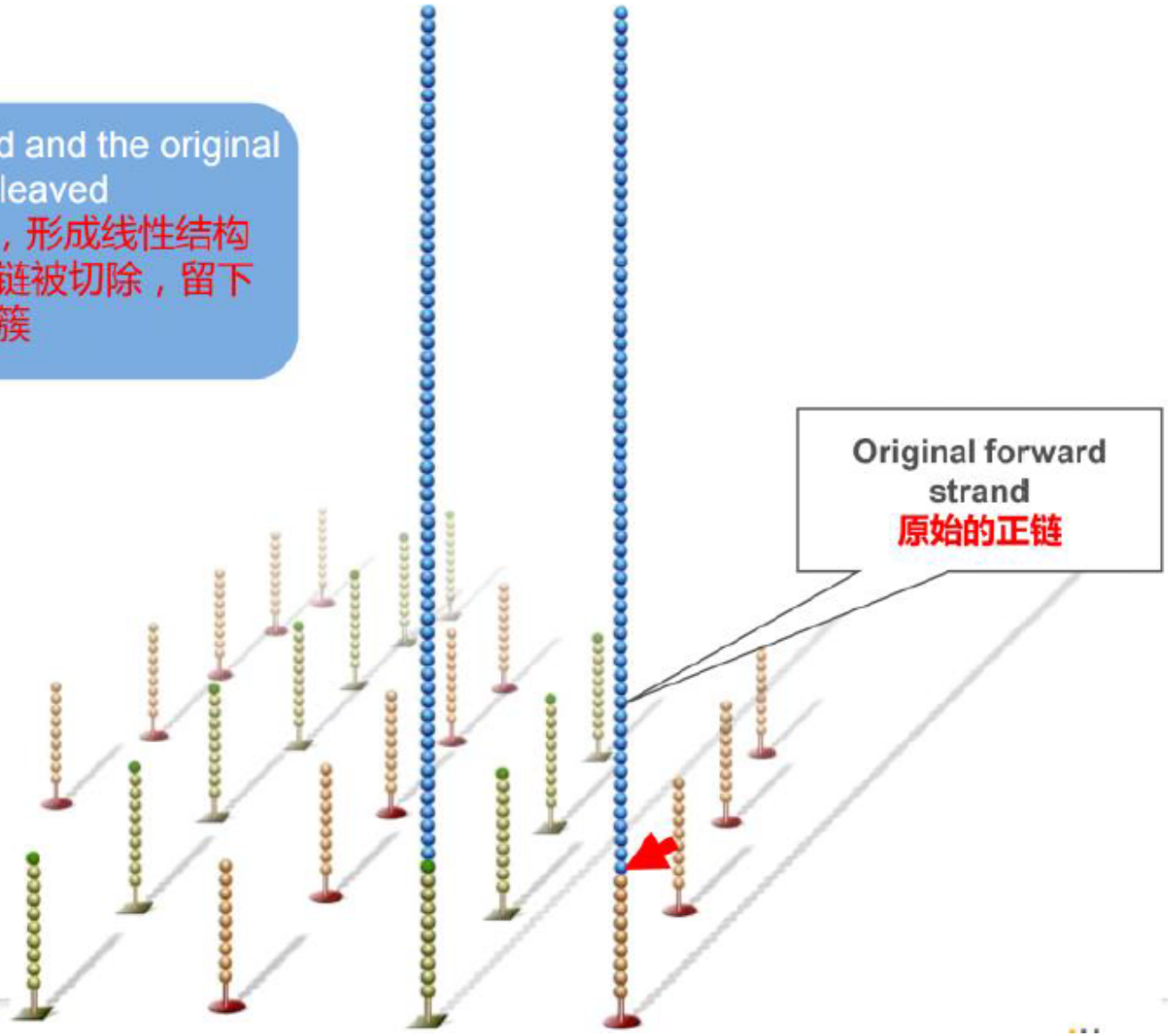


Paired End Sequencing
双末端测序



Paired End Sequencing
双末端测序

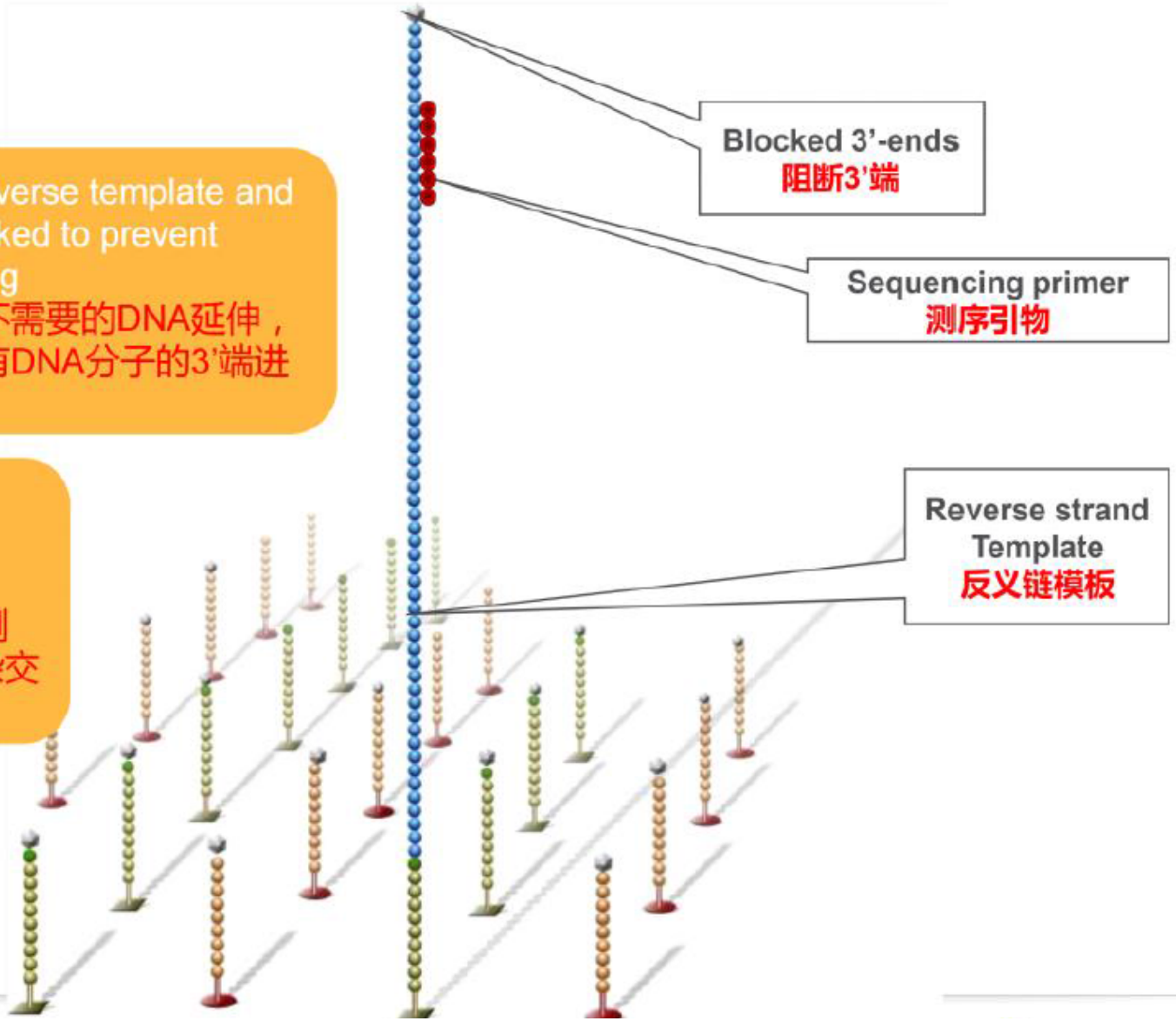
Bridges are linearized and the original forward template is cleaved
双链桥式结构被变性，形成线性结构的DNA簇；随后正义链被切除，留下只含有反义链的DNA簇



Paired End Sequencing
双末端测序

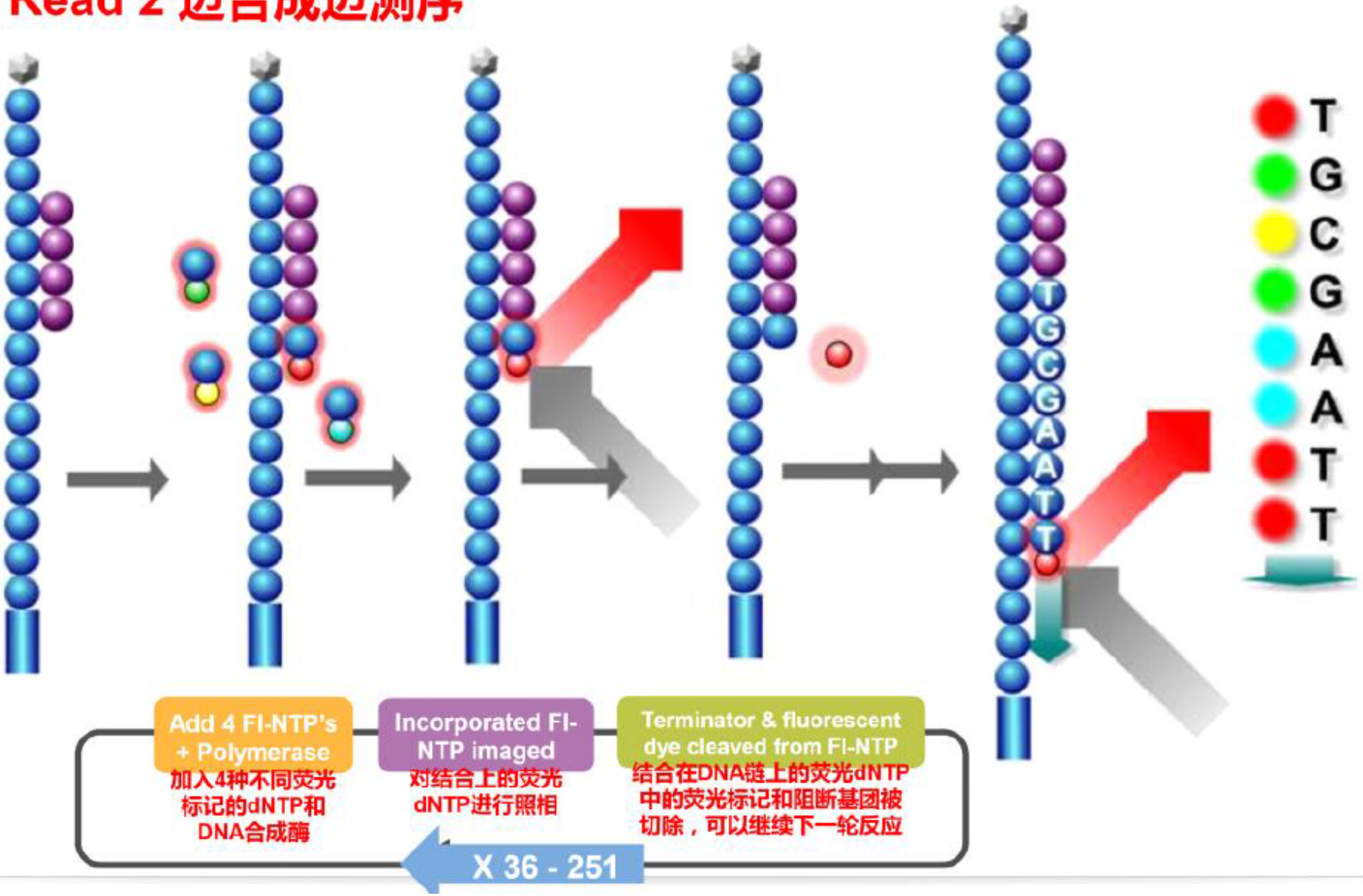
Free 3' ends of the reverse template and lawn primers are blocked to prevent unwanted DNA priming
为了防止测序过程中不需要的DNA延伸，对流动槽上结合的所有DNA分子的3'端进行封闭

Sequencing primer is hybridized to adapter sequence
Read 2测序引物与待测DNA分子的接头序列杂交结合

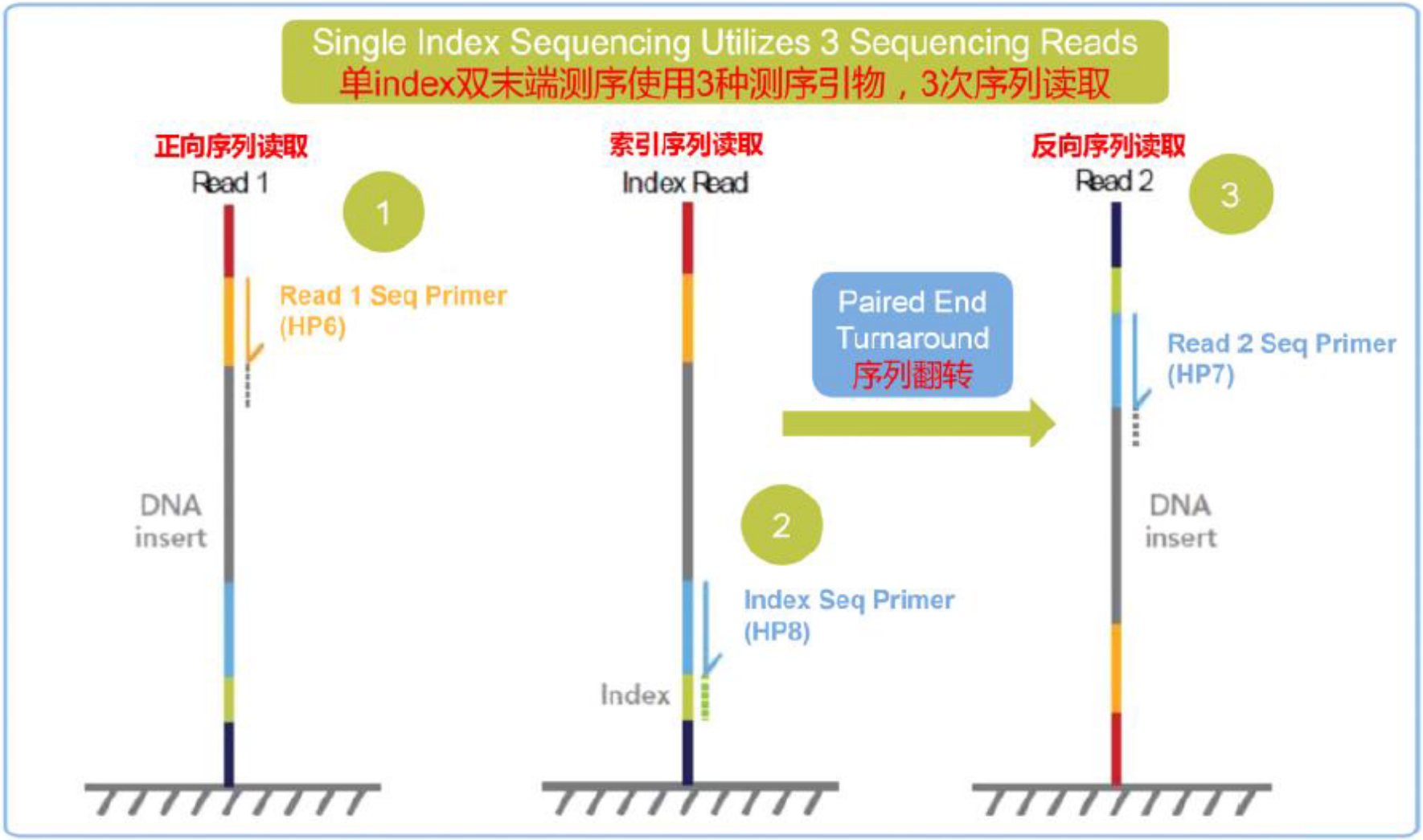


Sequencing By Synthesis 2nd Read

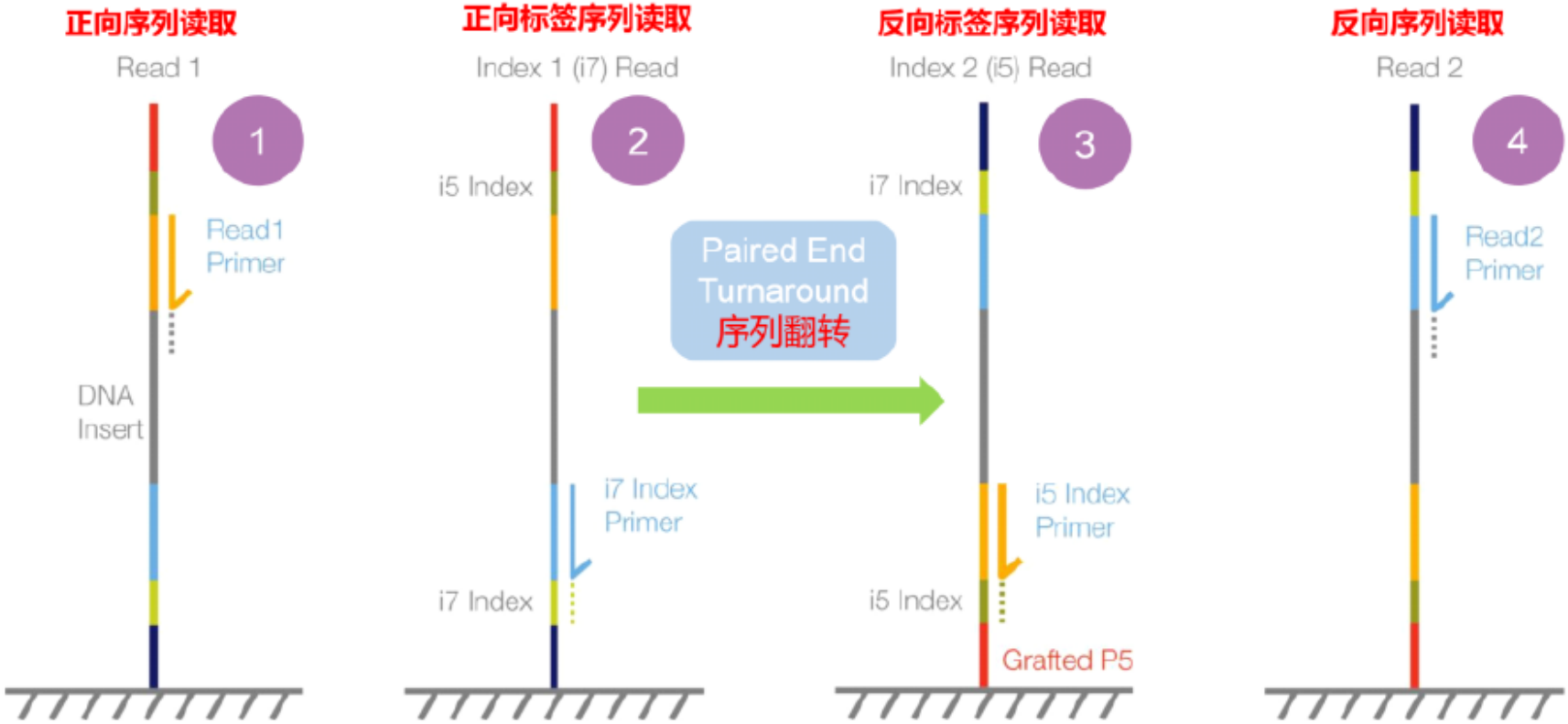
Read 2 边合成边测序



Sequencing with Paired Ends
双末端测序（单标签）



Sequencing Paired End Libraries with Dual Index Read
双末端测序（双标签）适用于NextSeq, HiSeq X/3000/4000



Dual Index Sequencing Utilizes 4 Sequencing Reads
双标签双末端测序包含4次测序读取，用到4条不同测序引物